

# A mutation in the gene of a glycolipid-binding protein (GM2 activator) that causes GM2-gangliosidosis variant AB

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Received 21 June 1991; revised version received 11 July 1991

GM2-gangliosidosis is a neurological disorder caused by a genetic deficiency of either the  $\beta$ -hexosaminidase A or the GM2 activator, a glycolipid binding protein. In a patient with an immunologically proven GM2 activator protein deficiency, a T<sup>412</sup> → C transition (counted from A of the initiation codon) was found in the coding sequence, which results in the substitution of Arg for the normal Cys<sup>107</sup> in the mature GM2 activator protein. The remainder of the coding sequence remained entirely normal.

GM2-gangliosidosis AB variant; GM2 activator protein; Mutation;  $\beta$ -Hexosaminidase; Ganglioside; Glycolipid-binding protein

## 1. INTRODUCTION

The GM2-gangliosidosis is a group of inherited disorders caused by an accumulation of ganglioside GM2 and a few related glycolipids mainly in the neurons of the patients (for review, see [1]). The lysosomal degradation of ganglioside GM2 requires a catalytically active  $\beta$ -hexosaminidase A, which consists of two subunits,  $\alpha$  and  $\beta$ , and in addition, a hexosaminidase A specific glycolipid binding protein, the GM2 activator.

Inherited defects in any of the three protein components in the system,  $\beta$ -hexosaminidase subunits  $\alpha$  and  $\beta$ , and the GM2 activator protein, thus may cause a GM2-gangliosidosis. Up to now, multiple genetic abnormalities in the  $\alpha$  and  $\beta$  subunits of the  $\beta$ -hexosaminidase have been identified. Mutations in the  $\alpha$ -chain lead to Tay-Sachs disease, while mutations in the  $\beta$ -chain are responsible for the failure of both  $\beta$ -hexosaminidase A ( $\alpha,\beta$ ) and B ( $\beta,\beta$ ) in Sandhoff's disease. GM2-gangliosidosis due to defective GM2 activator protein but with normal  $\beta$ -hexosaminidase isoenzymes is known as AB variant [1].

The information on the complete sequence of the cDNA coding for the normal human GM2 activator protein and the structural organization of its gene [2,3], made it possible to elucidate for the first time the genetic cause of the GM2 activator deficiency in a patient with GM2-gangliosidosis variant AB.

**Abbreviations:**  $\beta$ -hexosaminidase,  $\beta$ -N-acetyl-D-hexosaminidase, (EC 3.2.1.52); PCR, polymerase chain reaction

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## 2. MATERIALS AND METHODS

### 2.1. Patient

Cultured fibroblasts of a patient with proven GM2-gangliosidosis AB variant were used for the study [4]. The patient was a black female, who died at the age of 14 months. Diagnosis of GM2-gangliosidosis AB variant was established by a pathological accumulation of ganglioside GM2 in brain biopsy tissue, similar to classical Tay-Sachs disease, without a deficiency of  $\beta$ -hexosaminidase activities [4]. Recent immunological studies gave evidence for lack of GM2 activator protein in cultured fibroblasts of the patient [5].

### 2.2. RNA isolation and characterization

Total RNA was isolated from cultured skin fibroblasts by using the guanidine isothiocyanate/cesium chloride procedure [6]. Poly(A) RNA was selected using Type 7 oligo(dT) cellulose (Pharmacia, Freiburg). Northern transfer of electrophoresed mRNA onto nitrocellulose membranes was done according to Ausubel et al. [7].

### 2.3. Isolation of genomic DNA

Genomic DNA was isolated from cultured fibroblasts according to a method of Ausubel et al. [7].

### 2.4. PCR Amplification of cDNA and genomic DNA

First strand cDNA was synthesized from total RNA by specific oligonucleotide primers (Fig. 1), using the kit purchased from Promega (Madison, WI) according to the manufacturer's instructions. The entire protein coding sequence including 33 bases of the 5'-untranslated region and 96 bases of the 3'-untranslated region was amplified by PCR [8], using a series of synthetic 18-21-mer oligonucleotide primers (Fig. 1). The genomic fragment containing the mutation was amplified by PCR based on the known exon/intron structure of the gene (Fig. 1).

### 2.5. Direct sequencing of the DNA

The amplified DNA was purified by centrifuge-driven dialysis on a Centricon 30 microconcentrator (Amicon, Beverly, MA). Direct sequencing of the PCR-products was done by employing a Sequenase kit (United States Biochemical Co., Cleveland, OH) and the method of Wong et al. [9]. A total of five synthetic 18-21-mer oligonucleotide sequencing primers were used (Fig. 1).

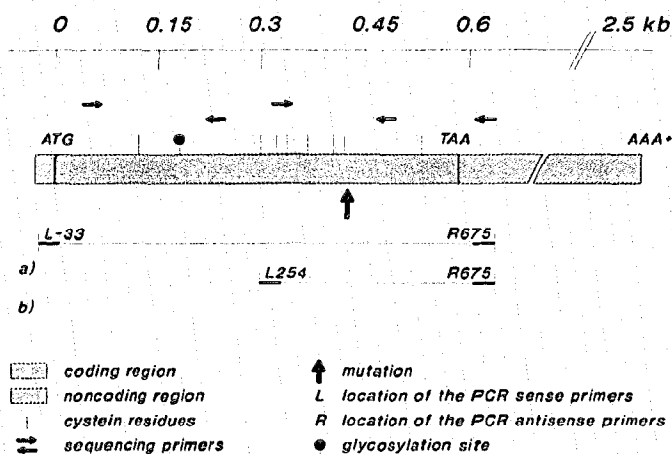


Fig. 1. Organization of the full length cDNA of the GM2 activator protein and location of the PCR and sequencing primers. The primer combinations used for amplification are shown above the bars representing the PCR products of cDNA (a) and genomic DNA (b). The position of the starting base of the PCR primer is numbered from the A of the initiation codon.

### 3. RESULTS AND DISCUSSION

Northern blotting experiments on the poly (A) RNA fraction showed the presence of the GM2 activator protein mRNA of apparently normal size (data not shown). cDNA analysis was done by PCR-amplification and direct-sequencing of the entire protein coding region. No abnormality in the cDNA sequence was found, except for a  $T^{412} \rightarrow C$  transition (numbering from the A of the initiation codon), which leads to a substitution of Arg for Cys<sup>107</sup> in the mature GM2 activator protein. This  $T^{412} \rightarrow C$  transition was observed in 4 different

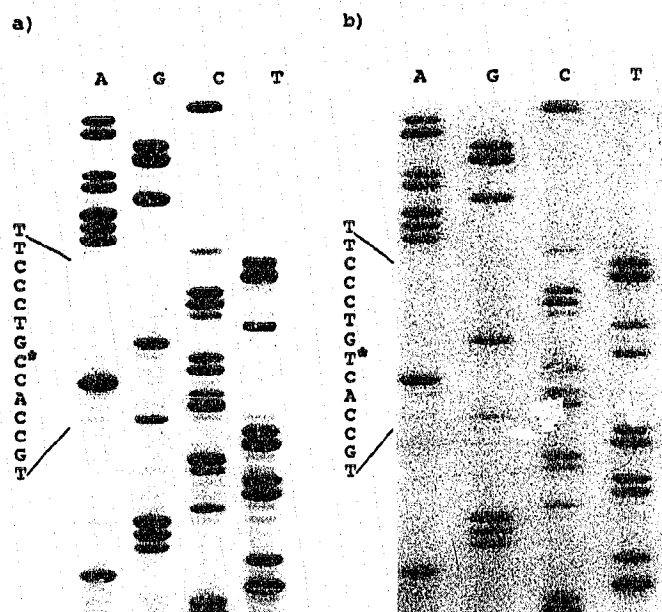


Fig. 2. Sequence of a portion of the GM2 activator protein coding strand. Direct sequence data of PCR products were obtained (a) from patient's mRNA and (b) from a control mRNA. The  $T \rightarrow C$  transition is indicated by a star.

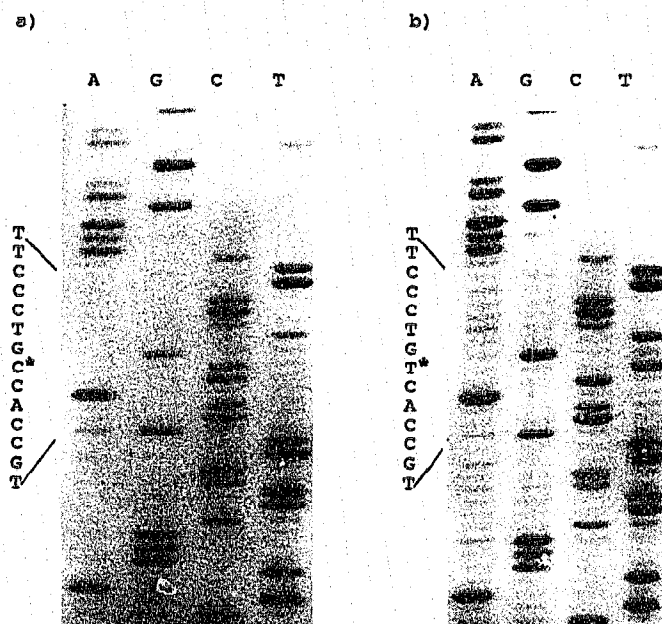


Fig. 3. The genomic sequence (a) of the patient's GM2 activator protein and (b) of a normal control. The  $T \rightarrow C$  transition is indicated by a star.

batches of cDNA, each prepared by amplification of different batches of cellular RNA (Fig. 2).

Amplification and sequencing of the genomic DNA around the mutation produced the mutated sequence each time (three times) (Fig. 3). Two assumptions are possible: first, the patient is homozygous for the identified mutation and second, one allele has the identified mutation and the other allele has a deletion in the areas of either or both of the primers. In either case, only the mutated protein is expressed in the patient's tissue.

The mutation eliminates the seventh of the eight Cys residues in the mature protein and thus can result in a failure to form normal disulfide bonds. The resulting destabilization of the normal three-dimensional structure could prevent the normal processing and transport to the lysosome and/or could make the mutant protein sensitive to proteolytic degradation. Either possibilities can explain the lack of GM2 activator protein in the patient's tissue.

**Acknowledgements:** We would like to thank Dr J. Weber for critical discussions, G. Weiß and A. Rath for excellent technical assistance in cell culture work and L. Kochhan for synthesizing the oligonucleotide primers. This work was supported by grants of the Deutsche Forschungs-gemeinschaft (SFB 284, C3), from the USA PHS (P30-HDO3110, RO1-NS24289 and RO1-NS28997) and an Alexander von Humboldt Senior Scientist Award, IV-A-97 (to K. Suzuki).

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